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(54) Title of the Invention: **A Cosmetic Material**

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Specification

1. Title of the Invention: **A Cosmetic Material**

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*Translator's Note: The legibility of the Japanese was poor. One critical item in the translation is in doubt as a result of this. The expression "lactobacillus fermentation solution" could be translated as "lactobacillus fermentation solution" or "lactobacillus bacterial fermentation solution". Unfortunately, there is one Japanese character that is too blurred to decipher. We believe we have rendered the translation accurately but because of the lack of clarity, some doubt exists. Problem areas are indicated with a [?] or as being [illegible].

2. Claim

1. A cosmetic material characterized in that it contains a Lactobacillus fermentation solution of soybean milk.

3. Detailed Description of the Invention

(Field of industrial use)

This invention relates to a cosmetic material that prevents oxidation of the skin and renders human skin white of which a Lactobacillus fermentation solution of soybean milk is the effective component.

[Prior art]

Cosmetic agents in which peroxides such as hydrogen peroxide and zinc peroxide are present have long been used for the purpose of removing blemishes such as liver spots and freckles that appear on the skin. However, because these peroxides are extremely unstable substances, there are difficulties in storing them and in compounding them with cosmetic material bases. In addition, their whitening effect is insufficient. Moreover, although cosmetic materials in which vitamin C, cysteine and colloidal sulfur are compounded have been used for the purpose of whiteness, their effectiveness is not adequate.

Whitening cosmetic agents in which kojic acid is used (Japanese Patent Announcement 56-18569 [1981]), melanin production inhibiting ointments in which kojic acid is used (Japanese Patent Announcement 61-10447 [1986]) and whitening cosmetic materials which contain kojic acid derivatives (Japanese Patent Announcement 61-60801 [1986], Japanese Patent Announcement 61-60802 [1986] and Japanese Patent Announcement 56-79616 [1981]) have been disclosed.

Further, skin beautifying and whitening cosmetic materials that contain placenta extracts (Japanese Patent Announcement 48-30370 [1973]) and topical agents for preventing melanin production containing vitamin E and kojic acid (Japanese Patent Application Early Disclosure No. 56-75421 [1981]) have been disclosed.

[Problems the invention is intended to solve]

Of the components that are in use in whitening cosmetic materials in conventional technologies, glutathione, cysteine and vitamin C are of poor stability and do not have sufficient melanin production inhibiting action in growing cells.

Kojic acid, flavonols and vitamin E are useful substances that inhibit melanin production in growing cells and that have a whitening effect. However, there are difficulties in the methods of preparation [?] of them.

[Means for solving the problems]

The inventors conducted repeated research on melanin production inhibiting action. In particular, studies were conducted on B16 cells originating from melanoma in mice. This invention was perfected by discovering that Lactobacillus fermentation solutions of soybean milk exhibit marked effectiveness in inhibiting melanin production in B16 cells and that they can be used effectively for treating such forms of chromopexy as blotches and for whitening of liver spots and freckles.

This invention is a cosmetic material that contains Lactobacillus fermentation solutions of soybean milk.

Lactobacillus fermentation solution of soybean milk, which is the effective component of this invention, is an aqueous extraction of soybeans and [contains] so-called soybean milk and actobacilli, for example, Streptococcus thermophilus, Streptococcus lactis, Lactobacillus delbrueckii, Lactobacillus bulgaricus, Lactobacillus casei, Lactobacillus acidophilus and Lactobacillus thermophilus.

The topical agent of this invention is obtained by standard preparation methods using Lactobacillus fermentation solution of soybean milk, which is the effective component, and bases, auxiliary agents and additives that are commonly used in the manufacture of cosmetic materials such as toilet water, creams and emulsions.

The content of the effective component in this invention should be 0.01 to 100% (weight), and, preferably, 0.1 to 30% (weight), relative to the total amount of the cosmetic material.

Next, we shall present the results of experiments on the melanin production inhibiting action and antioxidant capacity of this invention.

Experimental Example 1. Whitening action on cells.

a. Experimental method

Amounts of one-half, one-fourth and one-eighth of a 10 ml Lactobacillus fermentation solution of soybean milk obtained in Example of Manufacture 1 to be described subsequently were added to amounts of 10 ml of Eagle's MEM culture medium containing 10 V/V% of bovine fetal serum to make experimental groups A, B and C, respectively. A group to which test material was not added was established as the control group.

The experimental groups prepared as described above and the control group were inoculated with cultured B16 cells in amounts of 1.0×10^{3}[?], after which the materials were cultured for 5 days in a 5% CO_2 gaseous phase. The culture medium was replaced one time. After culturing, the cells were peeled off and were centrifuged (approximately 700 G). The degree of blackness of the centrifuged pellets was compared visually with that of the cells of the control group.

b. Experimental results

The results are shown below.

Table

Experimental group	Group A	Group B	Group C
Degree of whitening	4+ to ~ 5+	3+	1+ to ~ 2+

The symbol + indicates the degree of whitening. 5+: white; 4+: white to gray; 3+: gray; 2+: gray to black; 1+: black (slightly paler than control group); 0: black (control).

On the basis of the experiment described above, it was evident that the Lactobacillus fermentation solution was of superior effectiveness in whitening of B16 cells.

Experimental Example 2. Antioxidant capacity

a. Experimental method

The test materials indicated below were used in the evaluation.

1) Purified water (control)

2) Vitamin E (20 μM) (comparison)

3) Vitamin E (40 μM) (comparison)

4) Lactobacillus fermentation solution of soybean milk (material of Example of Manufacture 1) (0.2%) (cosmetic material of this invention)

5) Lactobacillus fermentation solution of soybean milk (material of Example of Manufacture 1) (0.4%) (cosmetic material of this invention)

The aforementioned test materials were used to prepare test solutions of 1.0 ml of 500 mM ethanol solution of linoleic acid, 10.0 ml of 0.1 M phosphate buffer solution at pH 7.0, 9.0 ml of ethanol and 5.0 ml of the aforementioned evaluation test materials.

These test solutions were allowed to stand for 9 days in a dark place at 37°C, after which changes in the peroxide value over time were determined by the iron rhodanide method. Specifically, 4.7 ml of 75% ethanol, 0.1 ml of antimony rhodanate (antimony thiocyanate) and 0.1 ml of 2 x 10^{2}[?] M 3.5% hydrochloride solution of ferrous chloride were added to 0.1 ml of test solution, and, after precisely 3 minutes, absorbance at 500 nm was determined.

b. Experimental results

The results are shown in Figure 1.

On the basis of the experiment described above, it was ascertained that the Lactobacillus fermentation solution of this invention had an excellent antioxidant capacity.

Next, we shall present examples of this invention and examples of manufacture of the Lactobacillus fermentation solution of soybean milk which is the effective component of this invention.

[Examples]

Example 1. Cream

2.00% of polyethylene glycol monostearate (406.0)*, 5.00% of self-emulsifying glycerol monostearate, 5.00% of stearic acid, 1.00% of behenyl alcohol and 10.00% of liquid paraffin were heated and dissolved. This solution was added to a solution obtained by heating and dissolving 0.20% of peroxybenzoic acid ester, 5.00% of 1,3-butylene glycol, 0.01% of disodium edetate, 10.00% of Lactobacillus fermentation solution of soybean milk (product obtained in Example of Manufacture 1) and 51.8% of purified water. The materials were then emulsified, stirred and cooled to make a cream.

Example 2. Emulsion

1.00% of polyethylene sorbitan monostearate (208.0), 0.50% of polyoxyethylene sorbitan tetraoleate (608.0), 1.00% of oleaginous glycerol monostearate, 0.50% of stearic acid, 0.50% of behenyl alcohol, 4.00% of avocado oil and 4.00% of glycerol trioctanoate were heated and dissolved. This solution was added to a solution in which 0.20% of p-oxybenzoic acid ester, 5.00% of 1,3-butylene glycol, 0.14% of xanthane gum, 0.01% of disodium edetate, 10.00% of Lactobacillus fermentation solution of soybean milk (the substance manufactured in Example 2 [sic] and 73.15% of purified water were heated and dissolved. The materials were then emulsified, stirred and cooled to make an emulsion.

Example 3. Toilet water

8.00% of polyoxyethylene hardened castor oil (608.0), 15.00% of ethanol, 0.10% of p-oxybenzoic acid ester, 0.10% of citric acid, 0.30% of sodium citrate, 4.00% of 1,3-butyelene glycol, 0.01% of disodium edetate, 20.00% of Lactobacillus fermentation solution of soybean milk (the substance manufactured in Example of Manufacture 2) and 42.49% of purified water were stirred uniformly and dissolved, with toilet water being obtained.

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*Translator's Note: The numerical value "406.0" is not explained in the Japanese patent. It may be the molecular weight of what would be a low molecular weight polymer.

Example 4. Cream pack

2.00% of polyethylene glycol monostearate (408.0), 5.00% of self-emulsifying glycerol monostearate, 5.00% of stearic acid, 0.50% of behenyl alcohol, 15.0% of squalane and 5.00% of cetyl octanate were heated and dissolved. This solution was added to a solution in which 0.20% of p-oxybenzoic acid ester, 5.00% of 1,3-butylene glycol, 0.01% of disodium edetate, 5.00% of Lactobacillus fermentation solution of soybean mild and 71.29% of purified water were heated and dissolved. The materials were then emulsified, stirred and cooled to make a cream pack.

% in the examples of this invention is wt % in all cases.

Example of Manufacture 1

Soybeans were washed with water and immersed in water overnight. Four time their volume of water was added to the soybeans immersed in water and they were pulverized to a paste in a mixer.

A small quantity of defoamed silicone was added and the mixture was heated for 3 minutes at 110°C. It was cooled and then filtered with flannel to obtain soybean milk.

This soybean milk was heated and sterilized for 20 minutes at 2 atmospheres and was cooled, after which the Lactobacillus delbrueckii was inoculated and culturing was carried out for 10 hours at 50 to 55°C. During culturing, the material was separated into curd and supernatant. Only the supernatant was collected and a Lactobacillus fermentation solution was obtained.

Example of Manufacture 2

Soybeans were washed with water and immersed in water overnight. Four times their volume of water was added to the soybeans immersed in water and they were pulverized to a paste in a mixer.

A small quantity of defoamed silicone was added and the mixture was heated for 5 minutes at 100°C. It was cooled, filtered with flannel and soybean milk was obtained. This soybean milk was heated and sterilized for 15 minutes at 120°C. It was then cooled, after which Streptococcus thermophilus was inoculated and culturing was carried out for 72 hours at 30 to 40°C.

During culturing, it was separated into curd and supernatant. The supernatant was collected and a Lactobacillus fermentation solution of soybean milk was obtained.

[Effect of the invention]

When the cosmetic material of this invention is applied to the skin, it is safe. There is no damage to the skin as a result of the action of the Lactobacillus fermentation solution of this invention. It prevents deposition of pigment from melanin, such as blotches, freckles and liver spots. Moreover, it is an extremely useful cosmetic material having an antioxidant action.

4. Brief Explanation of the Figure

Figure 1 is a graph of the results of experiments showing the antioxidant capacity of the cosmetic material of this invention.

In the figure, X indicates the control, Δ indicates vitamin E (40 μM) (control), O indicates the vitamin E (20 μM) (control), ▲ indicates the Lactobacillus fermentation solution of soybean milk (product of Example of Manufacture 1) (0.2)* (effective component of this invention) and ● indicates Lactobacillus fermentation solution of soybean milk (product of Example 1 of this invention) (effective component of this invention).

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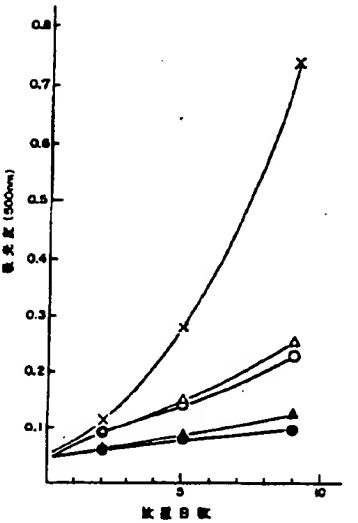
Figure 1



[vertical axis]: Absorbance (500 nm)
[horizontal axis]: Number of days allowed to stand

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*Translator's Note: The numerical value "0.2" is not explained in the Japanese patent. It probably should read 0.2%.

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⑭発明の名称　化粧料

⑳特　願　平1-263746
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明　細　書

1. 発明の名称　化粧料
2. 特許請求の範囲

1. 豆乳の乳酸菌醗酵液を含有することを特徴とする化粧料。

3. 発明の詳細な説明

〔産業上の利用分野〕

本発明は、豆乳の乳酸菌醗酵液を有効成分とする皮膚の酸化を防止し、人の肌を白くする化粧料に関する。

〔従来の技術〕

皮膚上に現れたしみ、そばかす等の斑点を除去するため、古くから過酸化水素、過酸化亜鉛等の過酸化物を配合した化粧料が使用されていた。しかしながら、これら　過酸化物は極めて不安定な物質であるため、保存性あるいは化粧料基剤への配合などに難点があり、その上色白効果も十分ではなかった。更に、ビタミンC、システイン、コロイド硫黄等を配合した化粧料が色白の目的で用いられるようになったが、その効果も十分満足するものではなかった。

更に、コウジ酸を用いた色白化粧料（特公昭56-18569号公報）、コウジ酸を用いたメラニン生成抑制用軟膏（特公昭61-10447号公報）、コウジ酸誘導体を含有する色白化粧料（特公昭61-60801号公報、特公昭61-60802号公報、特公昭56-19616号公報）等が開示されている。

更に、酵素抽出エキスを含有する皮膚美白化粧料（特公昭48-30370号公報）並びにビタミンE及びコウジ酸を含有するメラニン生成抑制外用剤（特開昭56-75421号公報）が開示されている。

〔発明が解決しようとする課題〕

従来の技術において、色白化粧料に用いられる成分のうち、グルタチオン、システイン、ビタミンCは安定性が悪く、かつ生きた細胞に対するメラニン生成抑制作用は不十分であった。

又、コウジ酸、フラボノール、ビタミンE等は生きた細胞に対するメラニンの生成を抑制するものであり、色白効果を有する有用な物質であるが、その製剤後に難点があった。

〔課題を解決するための手段〕

本発明者は、メラニン生成抑制作用について研究を重ね、 に細胞へのアクセスについてマウス黒色腫由来のB16細胞について検討を行い、豆乳の乳酸菌醗酵液がB16細胞のメラニン生成抑制効果を顕著に現すことを見出し、これを肝斑等の色素沈着症の治療並びに、しみ、そばかす等の色白化に使用し有効であることを見出し、本発明を完成した。

本発明は、豆乳の乳酸菌醗酵液を含有する化粧料である。

本発明の有効成分である豆乳の乳酸菌醗酵液は、大豆の水抽出液、所謂豆乳を乳酸菌例えば、ストレプトコッカス サーモフィラス(Streptococcus thermophilus) 、ストレプトコッカス ラクチス(Streptococcus lactis)、ラクトバチルス デルブリッキー (Lactobacillus delbrueckii)、ラクトバチルス ブルガリカス(Lactobacillus bulgaricus)、ラクトバチルス カゼイ(Lactobacillus casei) 、ラクトバチルス アシドフィラス(Lactobacillus acidophilus) 、ラクトバチルス サーモフィラス(Lactobacillus thermophilus)等である。

本発明の外用剤は、有効成分である豆乳 乳酸菌醗酵液を化粧水、クリーム、乳液 の化粧料の製造に通常使用される基剤、助剤、添加剤を使用し、通常の調製法によって得ることができる。

本発明の有効成分の含有量は、化粧料の全量に対し、0.01～100% (重量) 、好適には 0.1～30% (重量) である。

次に、本発明のメラニン生成抑制効果並びに抗酸化能の試験結果を示す。

試験例 1　　細胞の白色化作用

a. 試験方法

10V/V% 牛胎児血清を含むイーグルMEM 培地18 ml に下記製造例 1 で得られた豆乳の乳酸菌醗酵液10 ml の凍結乾燥物の1/2.1/4.1/8 をそれぞれ加えた試験区 A，B，C 並びに試料を加えない区をコントロール区とする。

以上のようにして調製した試験区及びコントロール区に培養B16細胞をそれぞれ1.0 ×10⁵ 個ずつ接種し、次37℃、5 % CO_2 気相下で 5 日間培養した。培地の交換はその間 1 回行った。培養後細胞を剥離し、遠心分離 (約700G) して細胞の遠心ペレットの黒色度をコントロール区の細胞と肉眼的に観察した。

b. 試験結果

下記の通りであった。

表

試 験 区	A区	B区	C区
白色化度	4＋～5＋	3＋	1＋～2＋

＋は白色化度を示す、5＋：白色、4＋：白色～灰色、3＋：灰色、2＋：灰色～黒色、1＋：黒色（コントロールより僅かに薄い）、○：黒色（コントロール）

以上の試験より本発明の乳酸菌醗酵液はB16細胞の白色化に極めて優れた効果を奏することが明らかである。

試験例 2　抗酸化能

a. 試験方法

評価試料として下記の試料を用いた。

1) 精製水（コントロール）

2) ビタミンE (20 μM) (対照)

3) ビタミンE (40 μM) (対照)

4) 豆乳の乳酸菌醗酵液（製造例 1 のもの)(0.2%)（本発明の化粧料）

5) 豆乳の乳酸菌醗酵液（製造例 1 のもの)(0.4%)（本発明の化粧料）

上記試料を500mM のリノール酸エタノール溶液 1.0ml 、PH 7.0の0.1M リン酸緩衝液10.0ml、エタノール 1.3ml及び上記評価試料 5.8mlの供試液を調製した。

各供試液を37℃の暗所で 9 日間放置した後、過酸化物価の経時変化をロダン鉄法により測定した。即ち、供試液 0.1ml に75% エタノール 4.7ml、30 %ロダン酸アンモニウム（チオシアン酸アンモニウム）0.1ml 、2 ×10⁻²M 塩化第一鉄の3.5%塩酸溶液 0.1ml を加え、正確に 3 分後に 500mm にお

ける吸光度を測定した。

b. 試験結果

第1図の通りであった。

以上の試験より本発明の乳酸菌醗酵液は極めて優れた抗酸化性を有することが明らかである。

次に、本発明の実施例並びに、本発明の有効成分である豆乳の乳酸菌醗酵液の製造例を示す。

〔実施例〕

例1　クリーム

モノステアリン酸ポリエチレングリコール（400.0）2.00%、自己乳化型モノステアリン酸グリセリン　5.00%、ステアリン酸　5.00%、ベヘニルアルコール　1.00%、流動パラフィン　10.00%を加温溶解する。この後、パラオキシ安息香酸エステル　0.20%、1.3-ブチレングリコール　5.00%、エデト酸二ナトリウム　0.01%、豆乳の乳酸菌醗酵液（製造例1で得られたもの）10.00%及び精製水51.8%を加温溶解した後に加え乳化、撹拌し、冷却してクリームとする。

例2　乳液

モノステアリン酸ポリオキシエチレンソルビタン(20E.O.)1.00%、テトラオレイン酸ポリオキシエチレンソルビット（60E.O）0.50%、精製豆モノステアリン酸グリセリン　1.00%、ステアリン酸　0.50%、ベヘニルアルコール　0.50%、アボカド油　4.00%、トリオクタン酸グリセリル　4.00%を加温溶解する。この後に、パラオキシ安息香酸エステル　0.20%、1.3-ブチレングリコール　5.00%、キサンタンガム　0.14%、エデト酸二ナトリウム　0.01%、豆乳の乳酸菌醗酵液（実施例2で製造したもの）10.00%及び精製水　73.15%を加温、溶解した後に加え、乳化、撹拌し、冷却して乳液を得る。

例3　化粧水

ポリオキシエチレン硬化ヒマシ油（60E.O.）1.00%、エタノール　15.00%、パラオキシ安息香酸エステル0.10%、クエン酸　0.10%、クエン酸ナトリウム　0.30%、1.3-ブチレングリコール　4.00%、エデト酸二ナトリウム　0.01%、豆乳の乳酸菌醗酵液（製造例2で製造したもの）30.00%、精製水

42.49%を均一に撹拌し、溶解して化粧水を得る。

例4　クリームパック

モノステアリン酸ポリエチレングリコール（400.0.）2.00%、自己乳化性モノステアリン酸グリセリン5.00%、ステアリン酸5.00%、ベヘニルアルコール0.50%、スクワラン　15.0%、オクタン酸セチル　5.00%を加温、溶解する。この後に、パラオキシ安息香酸エステル　0.20%、1.3-ブチレングリコール　5.00%、エデト酸二ナトリウム　0.01%、豆乳の乳酸菌醗酵液（製造例2で製造したもの）　5.00%　及び精製水71.29%を加温、溶解した後に加え、乳化、撹拌し、冷却してクリームパックを得る。

本発明実施例の%は、全て重量%である。

製造例1

大豆を水洗し、水に一夜浸漬する。この吸水大豆に4倍量　水を加えてミキサーでペースト状に粉　する。

これに消泡シリコーンを少量加え、110 ℃で3分間加熱する。冷却後、フランネルでろ過し、豆

乳を得る。

この豆乳を 130℃、2気圧で10分間加熱殺菌し、冷却後乳酸菌ラクトバチルス　デルブリッキーを接種して50～55℃で18時間培養を行う。培養時にカードと上澄みに分離されるので、上澄みを採取し豆乳の乳酸菌醗酵液を得る。

製造例2

大豆を水洗し、水に一夜浸漬する。この吸水大豆に4倍量の水をくわえてミキサーでペースト状に粉砕する。

これに消泡シリコーンを少量加え100　℃で5分間加熱する。冷却後、フランネルでろ過し、豆乳を得る。この豆乳を 120℃で15分間加熱殺菌し、冷却後ストレプトコッカス　サーモフィラスを接種し30～40℃で72時間培養を行った。

培養時にカードと上澄みに分離されるので上澄みを採取し、豆乳の乳酸菌醗酵液を得る。

〔発明の効果〕

本発明の化粧料は、これを皮膚に塗布すると、豆乳の乳酸菌醗酵液の作用により安全に何ら皮膚

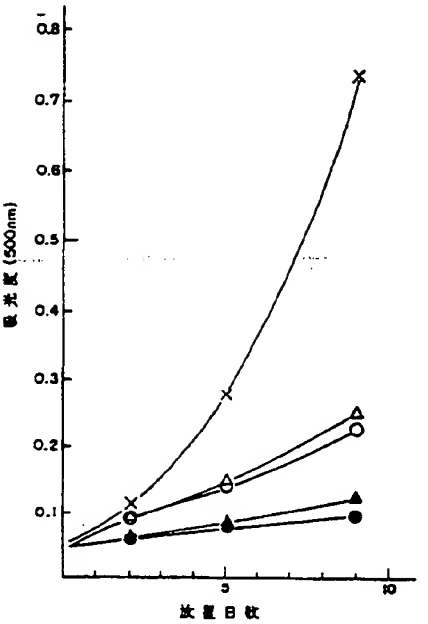
に毒害を与えることなく、しろ、そばかす、肝斑等のメラニンによる色素の沈着を防止し、更に抗酸化能を有する極めて有用な化粧料である。

4. 図面の簡単な説明

第1図は本発明の化粧料の抗酸化性を示す試験結果を現した図面である。

図中、×：コントロール，△：ビタミンE（40 μl）（対照）、○：ビタミンE（20 μl）（対照）、▲：豆乳の乳酸菌醗酵液（製造例1のもの）(0.2)（本発明の有効成分）、●：豆乳の乳酸菌醗酵液（製造例1のもの）（本発明の有効成分）それぞれ現す。

第 1 図



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